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PLANT-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Fern and bryophyte endozoochory by slugs

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Abstract Endozoochory plays a prominent role for the dispersal of seed plants, and dispersal vectors are well known. However, for taxa such as ferns and bryophytes, endozoochory has only been suggested anecdotally but never tested in controlled experiments. We fed fertile leaflets of three ferns and capsules of four bryophyte species to three slug species. We found that, overall, spores germinated from slug feces in 57.3 % of all 89 fern and in 51.3 % of all 117 bryophyte samples, showing that the spores survived gut passage of slugs. Moreover, the number of samples within which spores successfully germinated did not differ among plant species but varied strongly among slug species. This opens new ecological perspectives suggesting that fern and bryophyte endozoochory by gastropods is a so-far-overlooked mode of dispersal, which might increase local population sizes of these taxa by spore deposition on suitable substrates.

Keywords Dispersal · Gastropoda · Herbivory · Mutualism · Spore germination

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Introduction

In sessile organisms, such as plants, propagule dispersal is of particular importance. Many dispersal vectors of plant propagules are well known and there is a wealth of case studies on this topic (Schupp et al. 2010). Among dispersal mechanisms, endozoochory (transport of propagules inside animals) plays an important role for the dispersal of seed plants, and might even promote their germination (Beattie and Culver 1982; Duthie et al. 2006; Schupp et al. 2010). Similar to many vertebrates, gastropods have a broad diet spectrum including living and decaying plant material, fungi, dead animals, and seeds (Speiser 2001; Türke et al. 2012). Gastropod endozoochory has already been shown for lichens (which are not plants, but symbioses of photobiontic algae or cyanobacteria with fungi), first for the photobiont component (Fröberg et al. 2001) and recently also for complete lichens (Boch et al. 2011). However, whether gastropod endozoochory also occurs with plant taxa other than seed plants is not known. Cryptogams, such as ferns and bryophytes, are a very diverse group, and they inhabit essentially all terrestrial and some aquatic habitats (Porley and Hodgetts 2005; Kreft et al. 2010). Both ferns and bryophytes can reproduce sexually by gametangia (producing sperms and eggs) after spore germination, or vegetatively by producing propagules such as bulbils or gemmae, although vegetative reproduction is less common among ferns (McVeigh 1937; Raghavan 1989; Porley and Hodgetts 2005). Propagules can be dispersed by wind, water, or by attaching externally to animals, leading to exozoochorous dispersal (Kimmerer and Young 1995; Porley and Hodgetts 2005; Glime 2007). Although ferns and bryophytes are consumed by various animals, including gastropods (Davidson et al. 1990; Speiser 2001; Bråthen et al. 2007; Glime 2007; Arosa et al. 2010), there are only anecdotal reports that fern and bryophyte spores (Proctor 1961; van Tooren and During 1988; Davidson 1989; Bråthen et al. 2007; Arosa et al. 2010) and bryophyte fragments (Parsons et al. 2007) might survive animal gut passage. However, this has never been systematically tested in controlled experiments. Thus, the potential for endozoochorous spore dispersal of ferns and bryophytes by slugs has so far largely been overlooked. In addition, it is not known whether spores of different fern and bryophyte species differ in their ability to survive the gut passage of slugs, suggesting adaptation of some species to slug dispersal, as already shown for seed plants (Türke et al. 2012). Furthermore, it is not known whether slug species differ in their efficiency to disperse spores. This might, on the one hand, be the case because of different feeding preferences, e.g., that generalist species are better dispersers because of their wider diet spectra, or, on the other hand, by varying gut conditions allowing spore survival only among slug species that do not digest spores. In addition, the total amount of plant material, which is, in the case of ferns or bryophytes, related to the consumed amount of spores, will most likely differ among slug species. This might also affect the efficiency to disperse spores among slug species.

Therefore, we experimentally tested for endozoochorous spore dispersal by slugs. In particular, we tested whether (1) fern and bryophyte spores survive and germinate after passage through slug guts, (2) germination success of spores differs among fern and bryophyte species, and (3) slug species differ in how efficiently they disperse fern and bryophyte species.

Materials and methods

Ferns

Athyrium filix-femina (L.) Roth (Lady fern; Woodsiaceae) and *Dryopteris filix-mas* (L.) Schott (Male fern; Dryopteridaceae) are frequent species occurring throughout Central Europe and growing on various, mainly moderately humid soils in different forest types. *Gymnocarpium robertianum* (Hoffm.) Newman (Limestone oak fern; Woodsiaceae) occurs mainly in limestone areas throughout Central Europe in shaded to half-shaded, moderately humid conditions on scree slopes, in rock crevices, and wall cracks (Nebel et al. 1993).

Bryophytes

Bryum pallescens Schleich. ex Schwägr. (Tall-clustered thread-moss; Bryaceae) grows in humid or wet conditions on rocks and sand. It is widely distributed but locally rare in Central Europe. *Funaria hygrometica* Hedw. (Bonfire-moss; Funariaceae) and *Leptobryum pyriforme* (Hedw.) Wilson (Golden thread-moss; Bryaceae) are common, cosmopolitan species with a wide ecological amplitude growing mainly in open habitats. The liverwort *Pellia endiviifolia* (Dicks.) Dumort. (Endive pellia; Pelliaceae) grows on wet calcareous soils or limestone rocks distributed in limestone areas of the northern hemisphere (Frahm and Frey 2004).

Slugs

Arion vulgaris Moquin-Tandon (*A. lusitanicus* Mabille; Spanish slug; Arionidae) is an introduced species of wide ecological amplitude and omnivorous feeding behavior. As a consequence of its high reproductive rate, it is now very common in gardens and cultural land in Central Europe, where it has widely replaced the native slug species *Arion rufus* (L.) (Red slug; Arionidae). *Arion rufus* mainly occurs in forests, but also in open habitats, and has a broad diet spectrum. *Limax cinereoniger* Wolf (Ash-grey slug; Limacidae) inhabits different, near-natural forest communities and mainly feeds on decaying plant material, algae, and fungi (Turner et al. 1998; Speiser 2001). We chose these slug species because of their differences in distribution and ecological demands, and because they are among the largest gastropods in Central Europe, suggesting that they might be effective dispersers capable of moving greater distances than smaller species (Türke et al. 2010).

Collection of the involved species

In early summer 2011, we collected capsules of the four bryophyte species, fertile leaves of the three fern species, and 70 *A. vulgaris* individuals in the botanical garden of Bern (Switzerland; 46°57′N, 7°26′E). Totals of 70 *A. rufus* and 70 *L. cinereoniger* individuals were all collected at two forest sites in Thuringia (Germany), dominated by European beech. These were the Tautenburgerwald near the city of Jena (50°59′N, 11°41′E) and in the Hainich National Park near the city of Mühlhausen (51°50′N, 10°27′E).

Feeding experiment and cultivation of bryophytes and ferns

We performed a factorial experiment feeding ten replicates of each plant species to individuals of each slug species. The 210 slugs were kept individually in fauna boxes (180 × 135 × 65 mm) in a climate chamber (18/16 °C; 16/8 h light/dark cycle; Fig. 1a), where we first fed them for 72 h with tissue-paper to ensure defecation. Secondly, ten randomly selected individuals of each of the three slug species were fed for 48 h with fern and bryophyte material. Each slug individual was fed with either fertile leaflets of

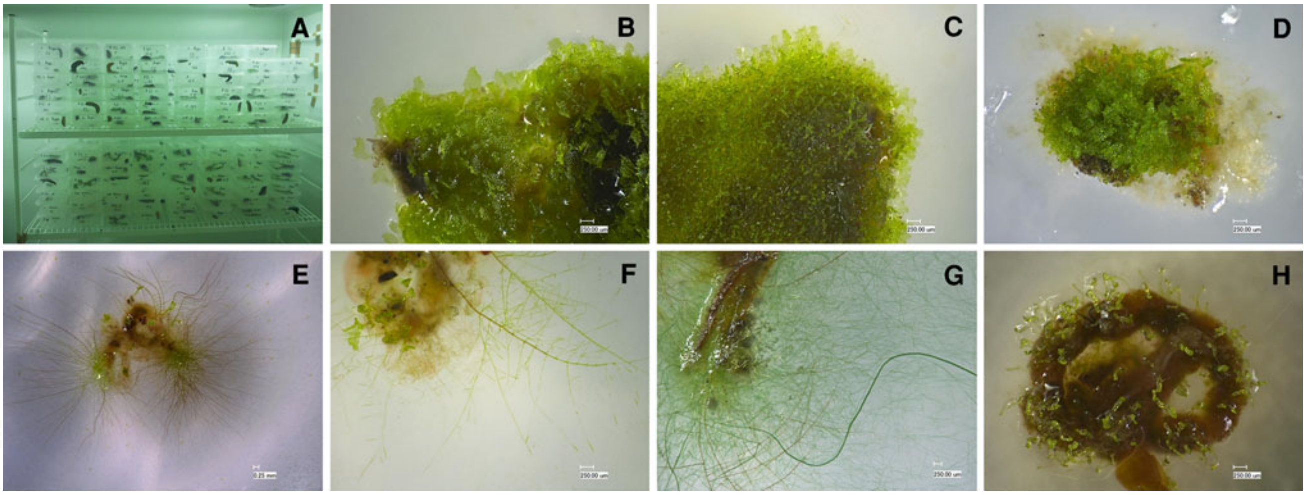

Fig. 1 **a** The feeding experiment showing the 210 fauna boxes with slugs in a climate chamber. **b–h** Microscope images showing prothallia of **b** *A. filix-femina*, **c** *D. filix-mas*, **d** *G. robertianum*, and protonema of **e** *B. pallescens*, **f** *F. hygrometica*, **g** *L. pyriforme*, and **h** *P. endiviifolia* germinated from slug feces

each of the three fern species or with approximately 30 capsules of each of the four bryophyte species. As a proxy for the consumed amount of spores, we roughly estimated the consumed amount of fern and bryophyte material using an ordinal scale (1: less than a third, 2: one- to two-thirds, 3: more than two-thirds). Then, we cleaned the slugs under running water to avoid secondary contamination with spores and transferred them into new fauna boxes with tissue-paper for defecation. After 48 h, we collected all fecal pellets of each slug individual from the fresh boxes to exclude contact of fecal pellets tested for spore germination with plant material. Fecal pellets were put in one separate Petri dish per slug individual onto phytagel (Sigma, USA), a culture medium without nutrients. Thus, each Petri dish represented one sample. From 28 June to 9 August 2011, we incubated fecal pellets in a climate cabinet (15 °C; 16/8 h light/dark cycle). Finally, we inspected all fecal pellets with a dissecting microscope and recorded germination of spores for each Petri dish; germination was visible as growth of bryophyte protonema or fern prothallia.

Statistical analysis

We analyzed numbers of samples with successful spore germination among plant species and among slug species with a generalized linear model. We also included the consumed amount of plant material as a covariate and the interaction between plant and slug species. Data were analyzed using R v.2.13.1 (R Development Core Team 2011).

Results

Overall, spores germinated from slug feces in 57.3 % of all 89 fern and in 51.3 % of all 117 bryophyte samples. On average, spores germinated in 60.0 % of samples of *A. filix-femina* ($n = 30$; Fig. 1b), 62.1 % of *D. filix-mas* ($n = 29$; Fig. 1c), 50.0 % of *G. robertianum* ($n = 30$; Fig. 1d), 44.8 % of *B. pallescens* ($n = 29$; Fig. 1e), 55.1 % of *F. hygrometica* ($n = 29$; Fig. 1f), 51.7 % of *L. pyriforme* ($n = 29$; Fig. 1g), and 53.3 % of *P. endiviifolia* ($n = 30$; Fig. 1h). These findings indicate that fern and bryophyte spores passed the slug guts without being digested and developed into juvenile bryophytes and ferns.

Table 1 Results of a GLM analysis, testing for differences in the number of samples in which spores germinated successfully among the seven plant and three slug species

	Number of samples with germination success		
	df	Deviance	*p*
Consumed plant material	2	9.415	0.009
Plant species	6	11.409	0.077
Slug species	2	76.698	<0.001
Plant species × slug species	12	20.002	0.067
Residuals	183	166.81	

The number of samples in which spores successfully germinated did not differ among plant species but varied strongly among slug species (Table 1; Fig. 2), with significantly lower mean values in *L. cinereoniger* samples (12.9 %) than in *A. vulgaris* (75.7 %) and *A. rufus* (74.2 %) samples. *Limax cinereoniger* did consume less plant material (in 97 % of all cases they consumed less than one-third of the material available) than *A. vulgaris* (56 %) and *A. rufus* (80 %). However, this does not seem to be the only reason for the observed differences among slug species because the slug species effect remained significant after correcting for the amount of plant material

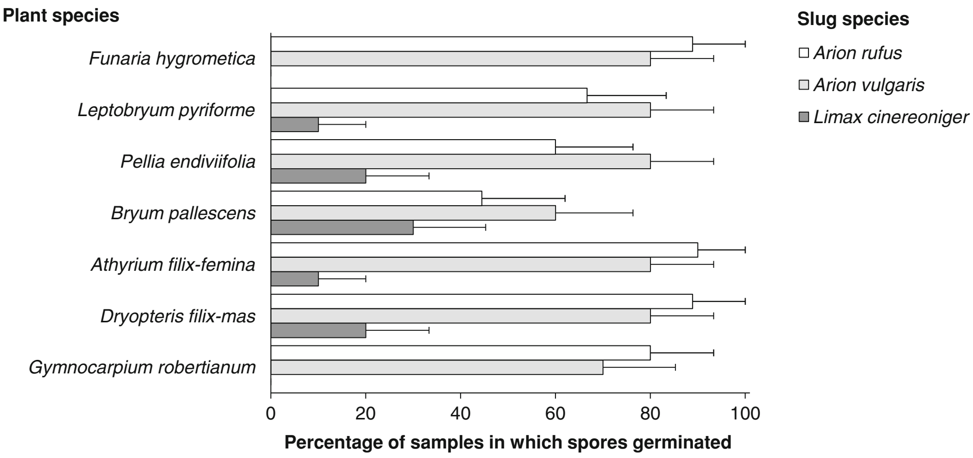

Fig. 2 The effect of plant and slug species on the percentage of samples in which spores successfully germinated (means + SEM)

consumed. In addition, the germination success in *L. cinereoniger* samples was still significantly lower than in samples of both *Arion* species. The results did not change qualitatively when analyzing the germination success of bryophyte and fern spores separately, except that the marginally significant plant species–slug species interaction became significant in the bryophyte-only dataset ($p = 0.016$; Fig. 2). This interacting effect reflects that the germination success of *B. pallescens* did not differ between *L. cinereoniger* and the *Arion* species, in contrast to the one of the other bryophytes.

Discussion

Gastropods are important herbivores of seed plants (Speiser 2001). Although they are also important consumers of bryophytes and ferns, this has rarely been documented (Davidson et al. 1990; Speiser 2001; Glime 2007), and endozoochorous dispersal of fern and bryophyte spores has only been suggested anecdotally and never tested in controlled experiments. For instance, Davidson (1989) reported better germination of the spores of two moss species, *Brachythecium rutabulum* (Hedw.) Bruch, Schimp & W.Gümbel and *Mnium hornum* Hedw., after gut passage through two *Arion* species. Furthermore, Proctor (1961) fed samples of the liverwort *Riella americana* M.Howe & Underw. to three domesticated mallard ducks and showed that liverwort spores can survive gut passage and germinate from duck feces. Fern and moss growth has also been observed from the feces of reindeer (Bråthen et al. 2007), earthworms (van Tooren and During 1988), and flying foxes (Parsons et al. 2007). Based on fern and moss growth from incubated feces samples, the authors proposed endozoochorous dispersal but did not test for it experimentally. In our controlled experiment, the spores of three fern and four bryophyte species survived passage through the guts of three slug species, without being digested, and germinated from slug feces. These results demonstrate that endozoochorous fern and bryophyte dispersal is possible.

The importance of fern and bryophyte endozoochory, relative to dispersal by water or wind, remains unknown. Endozoochorous spore dispersal by slugs is probably less important than wind for long-distance dispersal but might be more important for short-distance dispersal. Nevertheless, Türke et al. (2010) showed that *A. rufus* may endozoochorously disperse plant seeds up to at least 15 m. This is much farther than the dispersal distances measured by Kimmerer and Young (1995) for asexual brood branches of the moss *Dicranum flagellare* Hedw., which were dispersed externally on Philomycid and Arionid slugs (max. 23 cm, mean 3.7 cm). However, long-distance dispersal of fern and bryophyte spores by gastropods might be possible in some cases, as gastropods themselves can be dispersed by wind or animals (Rees 1965; Gittenberger et al. 2006; Wada et al. 2011).

In addition to dispersal, successful plant establishment requires firstly arrival at an appropriate site, and secondly nutrients to grow. We suggest that endozoochory by slugs may ensure both requirements: slug feces are sticky and could therefore attach spores to an appropriate substrate, and feces may even promote growth as they are rich in nutrients. Kimmerer and Young (1995) reported increased substrate adherence of moss fragments by slug secretions, in line with our suggestions for spores. Our results imply that, despite losses due to gastropod herbivory, local population sizes of bryophytes and ferns might be increased by endozoochorous spore dispersal, suggesting a mutualistic relationship between gastropods and these cryptogams.

In our study, the number of samples in which spores successfully germinated did not differ among plant species, despite differences between our experimental fern and bryophyte species in their ecological amplitudes and range sizes. This implies that endozoochorous dispersal of spores by slugs might be a general phenomenon among ferns and bryophytes. However, it remains open whether spore germination after slugs' gut passage would differ among fern and bryophyte species with even more pronounced differences in their ecological characteristics than among the plants we used in our experiment. This would suggest that the importance of endozoochorous spore dispersal by slugs might differ among habitats.

We found that fern and bryophyte germination success differed strongly depending on the slug species, with generally higher germination success for ferns and bryophytes regenerating from the feces of *Arion* species than for those regenerating after passage through *L. cinereoniger*. This may well be due to the wider diet spectra of the *Arion* species compared with *L. cinereoniger* (Turner et al. 1998; Türke et al. 2010). In our experiment, this was reflected in the generally lower amounts of plant material consumed by *L. cinereoniger*. However, the slug species effect was still present after correcting for the consumed amount of plant material in our analysis. The number of spores consumed may still differ among slug species, explaining part of the variability among them, but unfortunately we could not quantify the exact number of consumed spores. Thus, it remains unclear which slug species-specific factors, such as microbial or biochemical differences in gut microenvironment (Charrier and Brune 2003), affect the endozoochorous dispersal of spores.

Conclusions

We showed experimentally that fern and bryophyte spores can survive slug guts and germinate from feces, which so far had been largely overlooked. Given that gastropod herbivory on these taxa commonly occurs in nature, our findings imply that endozoochory of fern and bryophyte spores by gastropods is a so-far-overlooked mode of dispersal, which might increase local population sizes by spore deposition on suitable substrates.

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